Theoretical Problems in Modelling of Enzyme Sequences

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In vivo enzymes operate in enzyme systems[1] realizing reactions of metabolic networks. Using mechanisms of vectorial flow of matter and energy[2,3] as well as compartment and microcompartment of cells interior[4] the systems achieved high perfection[5] of catalytic action. Non-linear non-equilibrium thermodynamics describe the formation of coherent structures[6] of which enzyme systems are important example. Many of dynamic properties of the systems may be explained by control theory[7] or by more and more complicated kinetic models[8]. Measurable properties of the system reflect both properties of individual enzymes and the system as a whole and its environment. The system simultaneously realizes transport, binding and catalysis functions which are impossible to single out in the models. But rather than to bind or transport a substrate, the most important property of an enzyme is to catalyze the conversion of a substrate into a product[9]. On the other hand, the models in principle base on the approximation of an absolute continuous medium commonly used in most chemical models. This is reasonable for a large chemical system i.e. containing very large numbers of molecules but becomes doubtful for enzymes in conditions extant in vivo. What is even more important is that catalysis itself, viz. quantum mechanical interactions between a catalytic center c, a substrate s and a product p molecules is associated with the particular attributes of the objects c, s, p rather than with properties of an enzyme macrosystem and its environment.

New general models of enzyme systems, composed of elementary catalytic systems were suggested[10] for these reasons. A model of the elementary catalytic system (ECS), derived from the notions of the general theory od systems, which is capable of performing a single elementary catalytic act (ECA) is as simple as possible functional model of a system with the catalysis function only. The term „catalytic system" was first used by Rudenko[11] in his analysis of coupled catalysis and transport functions. To modelling of the optimum cooperation between many ECS´s and its environment the general models of multicatalytic system (MCS) were used[10,12]. The MCS´s were shown to contain finite numbers n of ECS. An effectiveness of an ECS action inside the MCS, described by a maximum classical probability (MPr(ECA)) of succesive ECA occurrence, increases non-linearly with increasing of n incomparison with a system of n ECS non-organized into MCS.For analysis of the catalysis function of enzyme systems the MCS models look to be very useful. It is just the purpose of the present paper to show that optimum value of n for simple MCS is about 15. In term of ECS and MCS a discussion of some modern biophysics and biotechnology problems will be made.

Elementary Catalytic System

The ECS is defined as a discrete, determinated system with purposeful behaviour which is a function of attributes of the system objects and bi-object relations. The purpose of the ECS action is to realize a sequence of bi-object events in a well-defined order (ECA). A set of the events does not contain those connected with flow of matter into and away of the ECS, which are function of environment attributes.

The ECS is composed (Figure 1) of a set of five objects which are represented by vertices of a structural graph and of the set of bi-object relations represented by the graph branches. The formal objects g and k where g is the source and k is the receiver of energy quanta simplify the modelling of energy flow inside ECS during ECA. Any object of ECS may exist in one of the states belonging to the object set of states, which is the subset of finite

minimal set of states required for ECA to occur. There are two attributes of a state $\underline{x}_i$ of object $\underline{x}$: I_{x_i} and E_{x_i}, where I_{x_i} is a constant time interval between the moments of appearance of two succesive active states (impulses) required for the reaction to occur, so called oscillation time in impulse-oscillation parametric model (IOM) of molecule[12], and E_{x_i} is the internal energy of object $\underline{x}$ in x_i state.

In ECS discrete time scale is used. All time parameters are assumed to be natural number counts of arbitrary time units. A bi-object relation (x_i, y_i) arises then and only then if a moment $SYN_{x_iy_j}$ of synchronization can be attained:

$$SYN_{x_iy_j} = a_{x_i} + n_{x_i} \cdot I_{x_i} = a_{y_j} + n_{y_j} \cdot I_{y_j} \qquad 1)$$

where: n_{x_i}, n_{y_j} are natural numbers; a_{x_i}, a_{y_j} are the moments of appearance of first active states in the system. The change of states $\underline{x}_i$, $\underline{y}_i$ of objects $\underline{x}$, $\underline{y}$ is the result of the relation.

To describe a flow of internal energy from $\underline{x}$ object (energy donor) to $\underline{y}$ object (energy acceptor) the following model was adopted:

$$E_{x_i} + E_{y_j} = E_{x_{(i+1)}} + E_{y_{(j+1)}} \qquad 2)$$

$$E_{x_i} > E_{y_j} \qquad 3)$$

$$E_{x_{(i+1)}} \geq E_{y_{(j+1)}} \qquad 4)$$

where: 2) follows from the energy concervation law; 3) and 4) define energy gradients before and after the flow, respectively.

The analysis of the general ECS model showed that an ECA process is the sequence of bi-object events (Figure 2) which occur if the conditions 1)-4) are fulfilled. Results obtained with computing of detailed particular ECS model (in preparation) showed, among others, that the catalysis effect (ECA duration time smaller that the time necessary for the relation ($\underline{s}$ $\underline{p}$) to occur) is possible to achieve for particular I_{c_o} values only and that the effect increases when the difference between I_{s_o} and I_{p_o} decreases.

Models of cooperation between ECS and its environment

To make many succesive ECA's occur, it is necessary to carry s_o and g_o into, and p_o and k_2 away from the ECS. Thus, the ECS model may be simplified and considered (Figure 3) a transformer of the chemical $\underline{s}$ and the energy $\underline{g}$ input signals into the respective signals $\underline{p}$ and $\underline{k}$.

The probability of the succesive ECA occurrence is a function of those events that depend on the interaction of the appropriate inputs/outputs to the ECS with its environment because the probabilities of all bi-object events inside the ECS are equal to unity (the determined system). For the estimation of MPr(ECA) the following simplifying assumptions are made:

- the number of objects in the ECS environment and the number of inputs/outputs with which ECS and its environment cooperate are determined
- the probability P(w) of any object appearance at any ECS input/output is the same
- the objects appear at appropriate moments $\underline{a}$ (eq. 1))
- individual events are independent
- any object $\underline{s}$, $\underline{p}$, $\underline{g}$, $\underline{k}$, $\underline{c}$ may exist in one of its equally probable states (Fig. 2).

1. An ECS inside stochastic environment
   The objects number = 4; the inputs/outputs number = 4
   $MPr(ECA)_1 \approx 2 \cdot 10^{-9}$
2. $\underline{n}$ ECS inside stochastic environment
   The objects number = 4n; the inputs/outputs number = 4n
   $MPr(ECA)_2 \approx 2 \cdot 10^{-9} \cdot n^{-8}$

3. Two ECS energy-coupled inside stochastic environment
First ECS serves as a source of energy quanta for the second. It is possible if:

$$ {}^{1}I_{k_2} = {}^{2}I_{g_o}; \qquad {}^{1}_{m}a_{k_2} = {}^{2}_{m}a_{g_o} \tag{5} $$

$$ {}^{2}I_{k_o} = {}^{1}I_{g_2}; \qquad {}^{1}_{m+1}a_{k_o} = {}^{2}_{m}a_{g_2} \tag{6} $$

$$ {}^{2}E_{g_o} = {}^{1}E_{k_2}; \qquad {}^{2}E_{g_2} = {}^{1}E_{k_o} $$

where the left superscripts and subscripts are ECS numerals in the system and ECA numerals in the ECS, respectively. From the energy flow model 2)-4) and 6) it follows[12] that ${}^{1}E_{c_o} > {}^{2}E_{c_o}$. If the number of objects and inputs/outputs are 6 then $MPr(ECA)_3 \approx 2 \cdot 10^{-8}$, in comparison with $MPr(ECA)_2 \approx 10^{-11}$ for $\underline{n} = 2$.

4. Two ECS chemically coupled inside stochastic environment
The $\underline{p}$ object in $\underline{p_o}$ state from first ECS serves as the $\underline{s}$ object in $\underline{s_o}$ state inside the second ECS. It is possible if:

$$ {}^{1}I_{p_o} = {}^{2}I_{s_o}; \qquad {}^{1}_{m}a_{p_o} = {}^{2}_{m}a_{s_o} \tag{7} $$

$$ {}^{1}I_{p_-} = {}^{2}I_{s_-}; \qquad {}^{1}_{m+1}a_{p_-} = {}^{2}_{m}a_{s_-} $$

$$ {}^{1}E_{p_o} = {}^{2}E_{s_o} \tag{8} $$

$$ {}^{1}E_{p_-} = {}^{2}E_{s_-} \tag{9} $$

States $\underline{p_-}$ and $\underline{s_-}$ serve to convey information of the lack of object $\overline{\underline{p}}$ and $\overline{\underline{s}}$ in the system. For the number of objects and inputs/outputs equal 6 $MPr(ECA)_4 \approx 2.5 \cdot 10^{-8}$, thus the same order as for model 3.

5. Chain MCS: $\underline{n}$ ECS energy-coupled inside stochastic environment.
   5a. The objects number $\underline{4n}$; the inputs/outputs number (2n+2)
   $MPr(ECA)_{5a} \approx 3 \cdot 10^{-4} \left[n(2n+2)\right]^{-2}$
   5b. The objects number (2n+2); the inputs/outputs number (2n+2)
   $MPr(ECA)_{5b} \approx 5 \cdot 10^{-3} (2n+2)^{-4}$
   The above formulas are true for $n \geq 3$, with terminal ECS disregarded. The absolute values of $MPr(ECA)_5$ decrease with increasing $\underline{n}$; $MPr(ECA)_{5a} \approx MPr(ECA)_1$ for n=12; $MPr(ECA)_{5b} \approx MPr(ECA)_1$ for n=18. The relative effectiveness $(MPr(ECA)_5/MPr(ECA)_2)$ increases with increasing $\underline{n}$, approximately with $\underline{n}^4$.

6. Chain MCS: $\underline{n}$ ECS chemically coupled inside stochastic environment.
   6a. The objects number 4n; the inputs/outputs number (2n+2)
   $MPr(ECA)_{6a} \approx 4 \cdot 10^{-4} \left[n(2n+2)\right]^{-2}$
   6b. The objects number (2n+2); the inputs/outputs number (2n+2)
   $MPr(ECA)_{6b} \approx 7 \cdot 10^{-3} (2n+2)^{-4}$
   The above formulas are true for $n \geq 3$, with terminal ECS disregarded. $MPr(ECA)_{6a} \approx MPr(ECA)_1$ for n=14; $MPr(ECA)_{6b} \approx MPr(ECA)_1$ for n=20.

7. Ring MCS: $\underline{n}$ ECS chemically coupled inside stochastic environment.
   7a. The objects number $\underline{4n}$; the inputs/outputs number $\underline{2n}$
   $MPr(ECA)_{7a} \approx 1 \cdot 10^{-4} n^{-4}$
   7b. The objects number $\underline{2n}$; the inputs/outputs number $\underline{2n}$
   $MPr(ECA)_{7b} \approx 4 \cdot 10^{-4} \cdot n^{-4}$
   $MPr(ECA)_{7a} \approx MPr(ECA)_1$ for $\underline{n} = 15$;
   $MPr(ECA)_{7b} \approx MPr(ECA)_1$ for $\underline{n} = 21$.

Discussion

The general character of the present considerations has prompted us to make the assumptions some of which need not to be obligatorily satisfied in individual cases. The MPr(ECA) estimates should be viewed as the orders of magnitude rather than exact values. On the other hand, the formal general analysis of ECS and MCS models leads to a number of conclusions which are well consistent with the modern biophysics and catalysis. At the same time it seems that the models have a good predictive capability helpful in studies of many biotechnology problems. From this point of view, for example, one should postulate a new look to be given to carriers employed in immobilization of enzymes in order to pick up carriers capable of an additional function of directional energy flow which could significantly enhance enzyme effectiveness; the relations 5)-6) or 7)-9) may be used in experiments with immobilization of bienzyme systems (the estimated effect due to chemical coupling is by at least one order of magnitude higher than established in experimental studies reported in literature); it seems that energetical relations[12] may help in studies of the formation of new series of chemical transformations in artificial non-biological MCS systems. However to achieve this, more detailed studies in the field are required.

Literature

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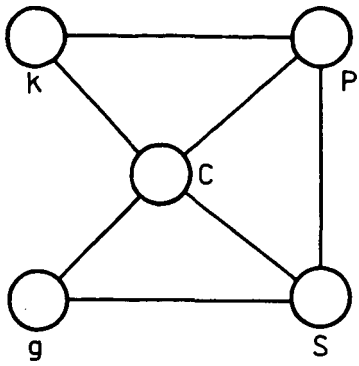

Figure 1. A graph of the ECS structure.

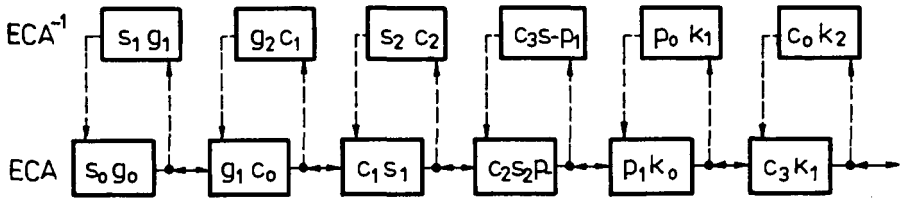

Figure 2. A diagrammatic presentation of an ECA.

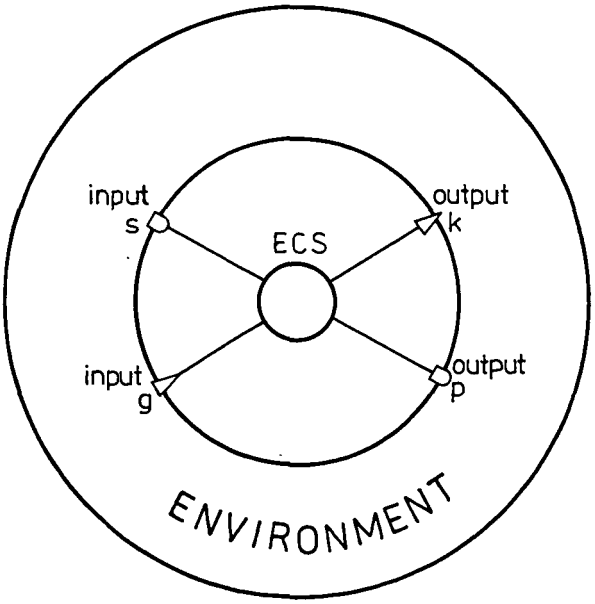

Figure 3. A simplified model of interaction between ECS and surroundings.